Ultraviolet Exposure as the Main Initiator of p53 Mutations in Basal Cell Carcinomas from Psoralen and Ultraviolet A-Treated Patients with Psoriasis

Hannes Seidl, Heidemarie Kreimer-Erlacher, Barbara Bäck, H. Peter Soyer, Gerald Höfler,* Helmut Kerl, and Peter Wolf

Department of Dermatology and *Institute of Pathology, Karl-Franzens University, Graz, Austria

Basal cell carcinoma, the most frequent skin cancer in humans, is often linked to chronic sun exposure. In psoralen and ultraviolet A-treated psoriatic patients, basal cell carcinomas may occur even more frequently; however, the exact etiology and mechanisms of tumorigenesis in psoriatic patients are unclear because psoralen and ultraviolet A is not only a carcinogen but also an immunosuppressor and because psoralen and ultraviolet A-treated psoriatic patients often have other (co)carcinogenic risk factors (e.g, therapeutic exposure to ultraviolet B, X-ray radiation, arsenic, tar, and/or chemotherapeutic agents such as methotrexate). In this study, we analyzed the DNA of 13 basal cell carcinomas from five psoralen and ultraviolet A-treated psoriatic patients for mutations of the *p53* tumor suppressor gene. DNA sequencing revealed a total of 11 mis-sense, two non-sense, and four silent mutations in seven of the 13 basal cell carcinomas (54%). Of the 13 total mis-sense or non-sense mutations, 12 (92%) occurred at dipyrimidine sites and nine (69%) were of the ultraviolet fingerprint type (eight C→T transitions and one CC→TT transition). Three of the C→T transitions occurred at dipyrimidine sites opposite a 5′-TpG sequence (a potential psoralen-binding site and target for psoralen and ultraviolet A mutagenesis). Thus, whether these mutations were induced by ultraviolet or psoralen and ultraviolet A was not clear. In addition, two other mutations (15%) occurred at 5′-TpG sites, one (8%) occurred at a 5′-TpA site (the most frequent site of psoralen binding and mutagenesis in cell and murine studies), and one (8%) involved a G→T transversion. These results suggest that (i) the major initiator of *p53* mutations in basal cell carcinoma in psoralen and ultraviolet A-treated psoriasis patients is environmental and/or therapeutic ultraviolet(B) exposure, and that (ii) psoralen and ultraviolet A itself causes only a smaller portion of *p53* mutations in psoralen and ultraviolet A-associated basal cell carcinomas.

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Psoriasis patients exposed to high cumulative doses of psoralen and ultraviolet A (PUVA) are at increased risk for cutaneous squamous cell carcinoma (SCC) (for review see Stern *et al*, 1998), and possibly for malignant melanoma (Stern *et al*, 1997). In addition PUVA-treated patients may be at increased risk for basal cell carcinoma (BCC) (Bruynzeel *et al*, 1991; McKenna *et al*, 1996). For instance, in the most recent follow-up report of the U.S. multicenter PUVA study there was a persistent strong UVA dose-dependent increase in the risk of SCC (by a factor of 17.6), but only a moderate increase in that of BCC (by a factor of 4.1) after adjustment for age, sex, and residence (Stern *et al*, 1998). Moreover, after adjustment for all other significantly associated risk factors (UVB radiation, tar, X-rays, and grenz rays), the risk of BCC was even less on sites other than the (sun-exposed) head and neck. At present, the exact reasons for the increased risk for skin cancer in PUVA-treated patients are not completely known because PUVA is not only mutagenic (Gunther *et al*, 1995) and carcinogenic (Kripke *et al*, 1982) but also immunosuppressive (Strauss *et al*, 1980; Kripke *et al*, 1982). The fact that many PUVA-treated psoriasis patients harbor additional potentially carcinogenic risk factors, such as exposure to UVB, X-rays, tar, methotrexate, and/or arsenic (Maier *et al*, 1996), makes the situation even more complex.

Recently, two groups of investigators (Nataraj *et al*, 1997; Wang *et al*, 1997) performed molecular sequence analysis studies on the *p53* tumor suppressor gene in PUVA-associated SCC in order to link mutation spectra to risk factors and identify the possible causes of that tumor type in long-term PUVA-treated subjects. Importantly, in both studies a large portion of the detected *p53* mutations (32% and 40%, respectively) were at dipyrimidine sites and of the UV fingerprint type (i.e, C→T and CC→TT transitions), suggesting that DNA damage caused by UV(B) exposure may be a significant factor in the formation of SCC in PUVA-treated patients. In the study by Nataraj *et al* (1997), however, approximately half of the mutations were detected at 5′-TpG sites, which are possible psoralen-binding sites and targets for PUVA mutagenesis. Indeed, comparison of the mutation

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Reprint requests to: Dr. Peter Wolf, Department of Dermatology, University of Graz, Auenbrugger Platz 8, 8036 Graz, Austria. Email: peter.wolf@kfunigraz.ac.at

Abbreviations: MOP, methoxypsoralen; BCC, basal cell carcinoma; SSCP, single-strand conformation polymorphism; SCC, squamous cell carcinoma.

spectra of SCC from PUVA-treated patients *vs* the general population revealed that significantly more PUVA-type mutations (defined as base changes occurring at 5′-TpA, 5′-TpG sites, and 5′-TpT sites) occurred in the former (64%) than in the latter (22%). Although it had previously been reported that repeated A–T sequences are hot spots for the photochemical reactions of 8-methoxypsoralen (8-MOP) with DNA and 5′-TpA sequences are the preferential sites for psoralen monoadduct or interstrand cross-link formation and subsequent carcinogenic events (Sage *et al*, 1993), the more recent work of Nataraj *et al* (1997) indicated that PUVA exposure may directly initiate a substantial proportion of skin cancers by causing *p53* mutations at psoralen-binding 5′-TpG sites.

To investigate the potential role of PUVA in BCC formation in psoriasis, we analyzed the *p53* gene (exons 4–9) in BCC from PUVA-treated psoriasis patients by polymerase chain reaction (PCR) –single-strand confirmation polymorphism (SSCP) analysis and DNA sequencing. The majority of *p53* mutations we detected in the BCC occurred at dipyrimidine sites and were of the UV fingerprint type; however, a smaller portion of mutations were at 5′-TpG sites. This suggests that even though the major factor in inducing *p53* mutations (and possibly BCC tumorigenesis) in PUVA-treated psoriasis patients seems to be therapeutic and/or environmental UV(B) exposure, PUVA itself also directly causes some of those *p53* mutations.

MATERIALS AND METHODS

Patients and BCC samples Material of 13 BCC was available from five psoriasis patients (A–E) who had a history of PUVA treatment. The exact patients' demographics, risk factors, PUVA data, and tumor characteristics are given in **Table I**.

DNA extraction For DNA extraction paraffin-embedded tumor samples were dissected to eliminate normal tissue adjacent to and in the tumors to reduce the portion of nontumor cells in the samples. Briefly, 7–10 μm tissue sections were deparaffinized with xylene (10 min) and ethanol (10 min), air dried, and then damped into deionized water. The dissected cells were then suspended in 50 μl of a solution containing 0.1 M Tris–HCl, pH 8.0, and 1 μg per μl proteinase K; incubated in a water bath at 55°C overnight; and then boiled for 10 min. The preparation was stored at –20°C until used.

PCR–SSCP analysis DNA from exons 4–9 of the *p53* gene was amplified using the primer sequences as listed in **Table II**. Primers with slight modifications were used to amplify exons 4 and 5 (Brash *et al*, 1991) and exons 6–9 (Wang *et al*, 1997). Four microliters of each lysate was used as the template in a 50 μl solution containing 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM deoxyribonucleoside triphosphate at 100 μM each, upstream and downstream primers 10 pM each, and 2.5 U AmpliTag Gold (Perkin Elmer, Vienna, Austria). PCR assays were run for 40 cycles of amplification as follows: denaturation at 94°C for 45 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. Before the first cycle, tubes were incubated for 12 min at 94°C, after the last cycle, for 7 min at 72°C. To avoid potential contamination of PCR and to rule out PCR-generated mutations, every PCR reaction included blank samples lacking DNA templates and other samples containing human placental DNA. After amplification, 50 μl of the PCR products was fractionated by electrophoresis in 2.5% MetaPhor agarose gel (FMC Bioproducts, Rockland, ME). Amplified and purified fragments were cut out of the gel and extracted using a commercially available gel extraction kit (Qiagen, Valencia, CA).

For SSCP, 0.5 μl (30 ng) amplified and purified DNA, 2.5 μl sample buffer, and 3 μl denaturing solution were denaturated at 95°C for 10 min and thereafter placed on ice. Samples were loaded on to mutation detection enhancement gels (Gene Gel Excel 12.5/24, Amersham Pharmacia Biotech, Vienna, Austria). The gels were then run for 95 min at 10°C (exons 7–9), 15°C (exon 6), and 20°C (exons 4 and 5). Running conditions were as follows: 600 V, 25 mA, and 15 W. Once run, gels were stained using a DNA Silver Staining Kit (Amersham Pharmacia Biotech). At least two independent PCR–SSCP analyses were carried out per tumor sample.

Nucleotide sequencing All amplified and purified samples were: (i) sequenced using 3.2 pM primer, 30 ng DNA, and sequencing reagents (Cycle Sequencing Ready Reaction; Perkin Elmer, Foster City, CA); (ii) precipitated; and (iii) separated in an ABI Prism 310 system (Perkin Elmer). Both DNA strands were sequenced, and mutations on one strand were always confirmed on the opposite strand. Sequence analysis was done on a Power Macintosh G3 (Apple Computer, Cupertino, CA) using Sequence Analysis Software (Perkin Elmer).

Statistical analysis Differences in the number of mutations types between different groups of subjects were analyzed for statistical significance using Fisher's exact test in the StatView statistical analysis

Table I. Patients' demographics, risk factors, PUVA data, and tumor characteristics

Subject	Sex[a]	Skin phototype[b]	Age at first PUVA treatment (years)	Total no. of PUVA treatments	Cumulative UVA dose (J per cm^2)	Psoralen	UVB therapy	Non-PUVA treatment risk factors	Total tumors	BCC of study	Location of BCC	BCC appearance after first PUVA treatment (months)
A	F	III	43	60	310	8/5-MOP	Yes	Tar, X-rays, methotrexate	7 BCC	A1	Scalp	129
										A2	Temple	220
										A3	Retroauricular	220
										A4	Retroauricular	189
B	F	N. A.	74	63	183	8-MOP	Yes	None	2 BCC, 7 SCC	B1	Retroauricular	1
										B2	Neck	119
C	M	III	47	813	4352	8-MOP	Yes	Arsenic, tar	1 BCC, 3 SCC	C1	Retroauricular	160
D	F	III	59	134	776	8-MOP	Yes	None	3 BCC	D1	Temple	154
E	F	III	50	452	1682	8/5-MOP	Yes	Arsenic, tar	5 BCC, 3 SCC	E1	Temple	182
										E2	Nose	241
										E3	Scalp	228
										E4	Scalp	186
										E5	Scalp	181
			Mean, 55 Range, 43–74	Mean, 304 Range, 60–813	Mean, 1460 Range, 310–4352							Mean, 170 Range, 1–241

[a]F, female; M, male.
[b]Skin phototype according to Fitzpatrick classification.

Table II. Primers used for PCR-SSCP and sequencing analysis of *p53*

Exon[a]	Primer direction[b]	Primer sequence (5′→3′)	Length of amplified fragment (BP)
4	F	AAT GGA TGA TTT GAT GCT GTC CC	274
	R	CTC AGG GCA ACT GAC CGT GC	
5	F	TTC AAC TCT GTC TCC TTC CT	229
	R	GCC CCA GCT GCT CAC CAT CG	
6	F	TGG TTG CCC AGG GTC CCC AG	224
	R	CGG AGG GCC ACT GAC AAC CA	
7	F	AGG CGC ACT GGC CTC ATC TT	177
	R	TGT GCA GGG TGG CAA GTG GC	
8	F	TTC CTT ACT GCC TCT TGC TT	186
	R	TTG CTT ACC TCG CTT AGT GC	
9	F	CAA GGG TGC AGT TAT GCC T	161
	R	ACT TGA TAA GAG GTC CCA A	

[a]Primers for exons 4 and 5 were selected as described by Brash *et al* (1991) and for exons 6–9 as described by Wang *et al* (1997).
[b]F, forward; R, reverse.

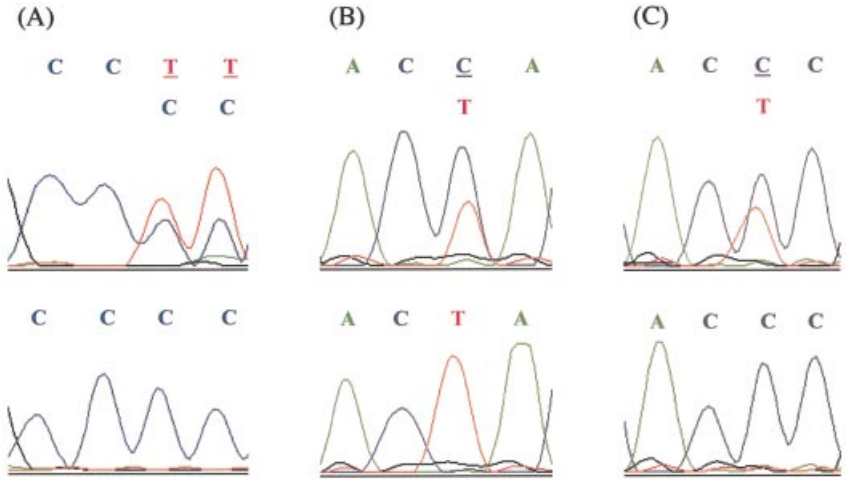

Figure 1 Sequence analysis reveals UV- and PUVA-type p53 mutations in PUVA-associated BCC. The upper panel shows electropherograms of DNA from (*A*)tumor A4 (exon 5, codon 177), CC→TT transition of UV type; (*B*)tumor E3 (exon 7, codon 234), T→C transition on a potential psoralen binding 5′-TpA:ApT site (i.e, of PUVA type); and (*C*)tumor A2 (exon 5, codon 155), C→T transition of UV type. The lower panel shows electropherograms of wild-type DNA from placenta.

program version 5.0.1 (SAS Institute, Cary, NC). $p \leq 0.05$ was considered significant.

RESULTS

Both UV- and PUVA-type *p53* mutations are present in BCC from PUVA-treated patients A total of 13 BCC were obtained from five PUVA-treated psoriasis patients and analyzed for *p53* mutations by PCR–SSCP analysis and sequencing of exons 4–9. Sequencing revealed a total of 17 mutations (11 mis-sense, two non-sense, and four silent mutations) in seven of 13 BCC (54%) (**Table III**). Mutations of the UV type [CC→TT transition (**Fig 1*A***) and C→T transition (**Fig 1*C***) at dipyrimidine sites] and the PUVA type (T→C transition at a 5′-TpA site) (**Fig 1*B***) were seen. Of the 13 mis-sense or non-sense mutations, 12 (92%) were at dipyrimidine sites and nine (69%) were of the UV type (eight C→T transitions and one CC→TT transition). Three of the C→T transitions were at dipyrimidine sites opposite a 5′-TpG sequence (a potential psoralen-binding site) (A1, codon 94; A3, codon 67; and D1, codon 317) (**Table III**). As this left the origin of these mutations (UV or PUVA) ambiguous, they were considered separately (**Fig 2**). In addition, two other mutations (15%) occurred at 5′-TpG sites (including one at a dipyrimidine and one at a nondipyrimidine site), one mutation (8%) at a 5′-TpA site (all of potential PUVA origin), and one involved a (G:C→T:A) transversion (8%). In addition, a BCC from patient C (tumor C1) showed a G→C transversion in exon 4 of codon 72, a known *p53* polymorphism (Olschwang *et al*, 1991). Two tumors (A1 and A3) had multiple mis-sense mutations (most of them of UV type) at exons 4 and 5, respectively (**Table III**). In support of a previous study (Nataraj *et al*, 1997), the greater intensity of wild-type *vs* shifted (i.e, mutant) bands in the PCR–SSCP analysis (data not shown) suggested that: (i) in most cases only one *p53* allele was mutated and the other was wild-type; (ii) only a portion of tumor cells harbored mutations; and/or (iii) contaminating normal cells were present in the tumor samples. Importantly, this is also supported by the observation that most of our electropherograms simultaneously showed wild-type and mutated DNA sequences (**Fig 1**), consistent with the SSCP results. Taken together, these findings suggested that there was no loss of heterozygosity at the *p53* gene of the BCC. Importantly, however, all *p53* mutations detected were somatic because they were not present in normal tissue samples from the patients.

BCC from PUVA-treated patients have a similar incidence of UV-type mutations but a higher proportion of PUVA-type mutations than BCC from the general population Data on the type and number of *p53* mutations in BCC from non-PUVA-treated subjects of the general population were gathered from several previous studies (Rady *et al*, 1992; Moles *et al*, 1993; Sato *et al*, 1993; Ziegler *et al*, 1993; Kanjial *et al*, 1995; Gailani *et al*, 1996; D'Errico *et al*, 1997; Ponten *et al*, 1997), pooled, and compared with the data on the mutations found in BCC from PUVA-treated patients (**Fig 2**). The total percentage of mis-sense or non-sense UV fingerprint mutations at dipyrimidine sites (i.e, C→T and CC→TT transitions) was 69% (nine of 13) [or 46% (six of 13), when not counting the three C→T mutations at 5′-TpG sites] in PUVA-treated patients and 64% (59/92) in the

Table III. Type of *p53* mutations detected in BCC from PUVA-treated psoriasis patients

Tumor	Exon	Codon	Base change	Surrounding sequence	Type of mutation[b]	Amino acid change(5′→3′)[a]
A1	4	75	**C→T**	g c c c **C** t g c a	UV	Pro→Leu
A1	4	88	**C→T**	c c a g **C** c c c c	UV	Ala→Val
A1	4	94	**C→T**	c t g t **C** a t c t	UV/PUVA	Ser→Leu
A1	5	144	**C→A**	c a g **C** t g c a c	Other	Gln→His
A2	5	155	**C→T**	g g c a c **C** c g c	(UV)	None
A2	5	165	**C→T**	a a g **C** a g t c a	PUVA	Gln→Stop
A3	4	67	**C→T**	a t g c **C** a g a g	UV/PUVA	Pro→Leu
A3	4	82	**C→T**	a c a c **C** g g c g	UV	Pro→Leu
A3	4	84	**C→T**	g c g g **C** c c c t	UV	Ala→Val
A3	4	88	**C→T**	c c a g **C** c c c c	UV	Ala→Val
A3	4	92	**C→T**	t g g c c **C** c t g	(UV)	None
A3	5	177	**C→T**	t g c c c **C** c a c	(UV)	None
A4	5	177	**CC→TT**	t g c c **C C** c a c	UV	Pro→Leu
D1	9	317	**C→T**	c c c **C** a g c c a	UV/PUVA	Gln→Stop
E2	7	229	**T→A**	g a c **T** g t a c c	PUVA	Cys→Ser
E3	4	122	**C→G**	a g t **C** a c a g a	(PUVA)	None
E3	7	234	**T→C**	c a c **T** a c a a c	PUVA	Tyr→His

[a]The 5-MOP, 5-Methoxypsoralen ′→3′ sequence is shown for the strand (transcribed or nontranscribed) containing the pyrimidine(s). Mutated bases are shown in bold. Multi- and dipyrimidine sequences are underlined. 5′-TpA:ApT and 5′-TpG:ApC sequences are double underlined.

[b]UV-type mutations were defined as C→T and CC→TT transitions at dipyrimidines. PUVA-type mutations were defined as mutations at potential psoralen-binding sites 5′-TpA:ApT and 5′-TpG:ApC. Silent mutations are shown in parenthesis.

general population. The difference was deemed not statistically significant when subjected to Fisher's exact test ($p \geq 0.2$); however, the percentage of *p53* mutations at 5′-TpG sites in BCC from PUVA-treated patients was statistically significantly higher than that in the general population [38% (five of 13) *vs* 2% (two of 92); Fisher's exact test, $p \leq 0.001$].

DISCUSSION

In this study, we examined the hypothesis that carcinogen-specific mutations are present in the *p53* gene of BCC from PUVA-treated patients. We found a total of 13 mis-sense or non-sense *p53* mutations in seven of 13 BCC (54%) and that 12 of these 13 mutations (92%) were at dipyrimidine sites and that nine (69%) of the mutations had the UV fingerprint (C→T or CC→TT transitions) (Brash *et al*, 1991). More important, the percentage of UV fingerprints in PUVA-associated BCC did not significantly differ from that in the general population (**Fig 2**). There are several possible explanations for this high proportion of UV fingerprint *p53* mutations. First, most BCC examined were from heavily sun-exposed body areas; on the other hand no PUVA-associated BCC from other body sites were available for study. Second, all patients in this study had a history of UVB treatment (a possible cause of UV-type *p53* mutations). Third, fluorescent tubes used for PUVA therapy in Europe emit a small portion of radiation within the UVB range (Nataraj *et al*, 1997), which may in turn cause UV-type mutations. Fourth, stable oxidation products of cytosine and thymidine induced by 8-MOP and UVA or by UVA alone could also induce C→T transitions (Wang *et al*, 1998). Finally, PUVA generates reactive oxygen species, which also may induce CC→TT transitions (Reid and Loeb, 1993). The hypothesis that exposure to PUVA or UVA alone may result in UV-type mutations, however, is strongly contradicted by results of cell culture (Sage *et al*, 1993; Chiou and Yang, 1995; Gunther *et al*, 1995) and murine (Nataraj *et al*, 1996) studies, in which UV-type mutations were rarely if ever observed after PUVA exposure.

An important finding in this study was that six of the 13 *p53* mutations identified (46%) were at potential psoralen-binding sites and thus may have been induced by PUVA; however, only one mutation (8%) was at a 5′-TpA site compared with five (38%) at 5′-TpG sites. This finding is similar to the results of a previous study (Nataraj *et al*, 1997) on PUVA-associated SCC, in which 13 of 25 *p53* mutations (52%) were found at 5′-TpG sites. Although cell culture (Sage *et al*, 1993; Chiou and Yang, 1995; Gunther *et al*, 1995) and murine studies (Nataraj *et al*, 1996) revealed that 5′-TpA sites are the most common targets for PUVA mutagenesis, a cell culture study (Gunther *et al*, 1995) using low (clinically relevant) doses of PUVA revealed that the highest percentage (approximately one-third) of mutations in murine fibroblasts containing *sup*F DNA occurred at 5′-TpG sites. Interestingly, in a study on PUVA-associated SCC our group of investigators found frequent mutation of the *Ha-ras* oncogene and a 28% incidence of mis-sense/non-sense mutations (five of 18) at 5′-TpG sites (Kreimer-Erlacher *et al*, in press). An alternative explanation for PUVA-induced mutations at 5′-TpG sites was recently proposed namely, reactive oxygen species-mediated damage at G, C, and/or T sites (Wang *et al*, 1998; Peritz and Gasparro, 1999).

Because molecular epidemiology studies can link mutations to causative agents, they are a potent tool for determining the factors that contribute to cancers (Peritz and Gasparro, 1999). In the case of PUVA-associated skin cancer, however, the situation is made very complex by the concomitant presence of multiple risk factors such as exposure to potentially carcinogenic and cocarcinogenic agents including UVB, X-ray radiation, medical tar, and methotrexate (Maier *et al*, 1996). For instance, two patients in the present study (C and E) had been exposed in the past to therapeutic arsenic, a well-known carcinogen that causes skin cancer after long latency periods. We found two PUVA-type mis-sense mutations (one at a 5′-TpA site and one at 5′-TpG site) in the six BCC samples from those patients but no mutations of any other type; however, arsenic exposure may lead to G→T transversions via the production of reactive oxygen species (Shibutani *et al*, 1991). Interestingly, in another study, we had found two C→T transitions and one G→T transversion in the *p53* gene of a total of four BCC from two arsenic-exposed, non-PUVA-treated patients (Seidl *et al* unpublished results). G→T transversions, however, can also be caused by exposure to UV-induced singlet oxygen via production of 8-hydroxyguanosine (Cheng *et al*, 1992), to benzo[*a*]pyrenes (e.g, from tobacco smoke) (Chiba *et al*, 1990), or to hydrogen radicals generated by γ-radiation (Braun *et al*, 1993). Patients A, C, and E in our study all had a history of medical treatment with tar, which may contain carcinogenic compounds, such as benzo[*a*]pyrenes (Godschalk *et al*, 1998). Indeed, one tumor (A1) harbored at exon 5 (codon 144) a G→T (G:C→T:A) transversion. Patient A had also received X-ray radiation on the scalp and oral

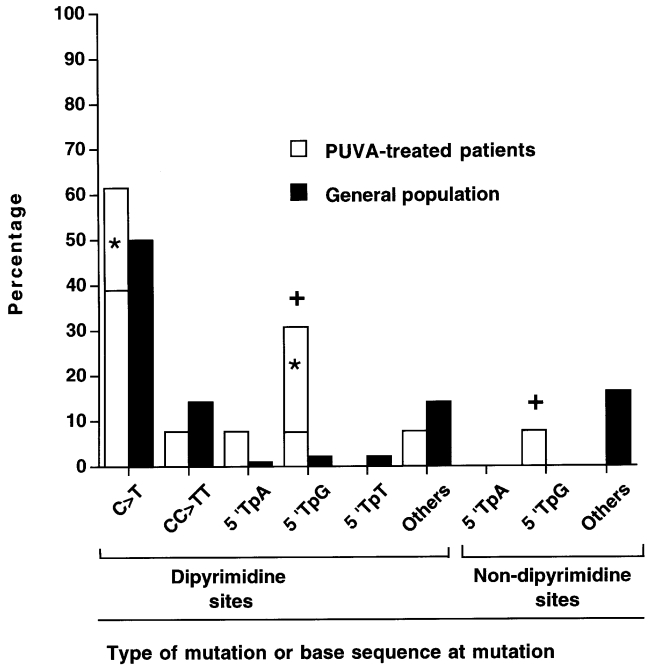

Figure 2. BCC from PUVA-treated patients have a higher proportion of mutations at 5′-TpG sites (i.e, mutations at potential psoralen-binding sites and thus potentially of PUVA origin) than do BCC in the general population. *p53* mutations detected in BCC from the general population in several previous studies (for details see *Materials and Methods*) were pooled and compared with those in BCC from PUVA-treated patients in terms of type and number. *Note that there were three C→T transitions at dipyrimidine sites, each of which occurred opposite a 5′-TpG sequence (a potential psoralen-binding site) (see **Table III**). In this graph, these mutations are represented at the top of the column marked C→T and at the top of the column marked 5′-TpG at dipyrimidine sites. The origin of these mutations (UV or PUVA) was ambiguous, and thus they were considered separately in the mutation spectrum analysis. +The proportion of *p53* mutations at 5′-TpG sites was statistically significantly higher in BCC from PUVA-treated patients than in those from the general population [38% (five of 13) *vs* 2% (two of 92), respectively; Fisher's exact test, $p \leq 0.001$].

methotrexate for the treatment of psoriasis. It is well-known that X-ray treatment can cause BCC, possibly by causing G→T mutations (Braun *et al*, 1993).

Two BCC (A1 and A3) had multiple mis-sense mutations, mostly of the UV type (**Table III**). As sequencing and PCR–SSCP were carried out on each exon separately and vector cloning was not performed in our study, it was therefore impossible to determine whether the mutations in the different exons of the tumors occurred in the same *p53* allele or in different alleles. Interestingly, in this context, a previous study by Ziegler *et al* (1993) revealed that 56% of human BCC contained *p53* mutations and that 45% of the tumors contained a second point mutation on the other *p53* allele. Consistent with the results of our study, however, D'Errico *et al* (1997) previously reported that all *p53* mutated BCC they studied apparently retained the wild-type *p53* allele suggesting that only one *p53* allele seems to be inactivated in BCC. Another previous study found multiple *p53* mutations in eight of 12 PUVA-associated human SCC (Nataraj *et al*, 1997). In a study of PUVA-induced SCC in mice, some tumors harbored multiple *p53* mutations (Nataraj *et al*, 1996). Indeed, the presence of multiple *p53* mutations in cancers suggests that clones harboring an initial mutation on one allele are targets for a second mutation on the other allele or that these mutations may arise independently, perhaps in different clonal subpopulations during tumor development (Nataraj *et al*, 1996). Indeed the latter hypothesis is supported by our finding that some BCC (A2, A3, and E3) had silent mutations (i.e, no change in amino acid sequence) of the UV or PUVA type (**Table III**) and that at least in some cases only a portion of tumor cells harbored *p53* mutations (**Fig 1**). Clearly, as was the case with the PUVA-associated SCC studied by Nataraj *et al* (1997), not all *p53* mutations in tumors may contribute to tumor development as some mutations may arise after tumor initiation by repeated exposure to agents such as PUVA and/or UV(B).

Our present results are also important because they contradict the previous finding of Greenblatt *et al* (1994) that approximately 90% of all *p53* mutations in cancers occur between exons 5 and 8. In contrast we found that: (i) seven of 13 *p53* mis-sense/non-sense mutations (54%) occurred in the nonconserved domain of exon 4 (codons 67–94); (ii) three (23%) in the conserved domain III of exon 5 (codons 144–177); (iii) two (15%) in the conserved domain IV of exon 7 (codons 229 and 234); and (iv) one (8%) in the nonconserved domain of exon 9 (codon 317). In previous studies, pooling of DNA sequencing results revealed that BCC appeared to have a major mutational hot spot between codons 241 and 280 and a minor region between codons 161 and 200 (Nataraj *et al*, 1995; references cited therein). In this study, we observed two mis-sense mutations (i.e, C→T transitions at a multipyrimidine site) at codon 88 of exon 4 (tumors A1 and A3), but nowhere else a hotspot mis-sense mutation incidence on the *p53* gene. Mutations in conserved domains, of the *p53* gene have been suggested to destabilize the *p53* protein (Cho *et al*, 1994), but little is known about mutations outside the conserved core domain.

In conclusion, our study revealed that the majority of *p53* mutations in BCC from PUVA-treated patients occurred at dipyrimidine sites and had the UV fingerprint, similar to the case in the general population (**Fig 2**); however, a smaller percentage of the mutations occurred at 5′-TpG sites, suggesting that even though the major cause of *p53* mutations (and possibly BCC tumorigenesis) seems to be environmental and/or therapeutic UV(B) exposure, PUVA itself may directly cause some *p53* mutations as well. Moreover, as only about half of the BCC in the present study harbored *p53* mutations, it may be that other tumor suppressor genes and/or oncogenes are targets for PUVA-associated damage leading to BCC tumorigenesis. One particular candidate that needs to be investigated is the "patched" gene, which is frequently mutated in BCC of patients with the basal cell nevus syndrome and in the general population (reviewed in Wikonkal and Brash, 1999). This study, however, highlights the value of the new field of molecular epidemiology as the results confirm on the molecular level those of many conventional epidemiologic PUVA follow-up studies, suggesting that PUVA itself may not be the major factor in BCC formation in PUVA-treated subjects (Stern *et al*, 1998).

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